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its ability to fine tune Smad activation. It will be exciting to learn more about how the expression and regulation of PPM1A influences TGFβ signaling in different physiological and pathophysiological contexts.

It is clear that PPM1A plays a crucial role in TGFβ signaling by regulating the level of R-Smad phosphorylation, but the specificity and selectivity of PPM1A for R-Smads remains to be seen. Lin et al. (2006) report that within the R-Smad molecules, PPM1A appears to show specificity in dephosphorylating the SXS motif, as phosphorylated Ser212 of Smad3 (a target of Cdk4) is not dephosphorylated by PPM1A (Lin et al., 2006). However, there is evidence that PPM1A may also target other pathways or proteins, including the p38 and PI3K pathways, that interact with or can be activated by TGFβ in certain contexts (Takekawa et al., 1998; Yoshizaki et al., 2004). Despite this, induction of p38 or Akt phosphorylation by TGFβ is not reduced by PPM1A in Mv1Lu or NIH 3T3 cells (Lin et al., 2006). Although the major role of PPM1A may be to impinge on TGFβ-regulated Smad signaling, it nonetheless appears certain that PPM1A has other targets and functions. These other functions may be concordant with the downstream effects of PPM1A-mediated dephosphorylation of Smad2/3, or they may be completely independent of the effects of PPM1A on Smad2/3. Teasing apart how these various pathways may be connected or regulated independently by PPM1A will be an interesting challenge for the future.

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Bone Formation: The Nuclear Matrix Reloaded

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In this issue of *Cell*, Grosschedl and colleagues (Dobreva et al., 2006) report that the nuclear matrix protein Satb2 represses *Hoxa2* expression and acts with other regulatory proteins to promote osteoblast differentiation. This work suggests a molecular mechanism that enables the integration of patterning and differentiation during bone formation.

Organogenesis depends upon a well-ordered series of events involving coordination of the molecular pathways that regulate the generation and patterning of specific cell types. A key question is whether regulatory networks for cell differentiation and patterning overlap or are separate processes. Skeletal development is an excellent context for investigating this complex problem because of the wealth of information emerging on molecular mechanisms that govern both skeletal differentiation and patterning from studies of model organisms and human mutations (Karsenty and Wagner, 2002). For example, members of the Hox homeodomain family of transcription factors are major regulators of skeletal patterning, whereas the Runt domain protein Runx2 is a master regulator of transcription that controls osteoblast differentiation. The patterning of skeletal elements and bone formation are generally thought to represent distinct pathways; how-

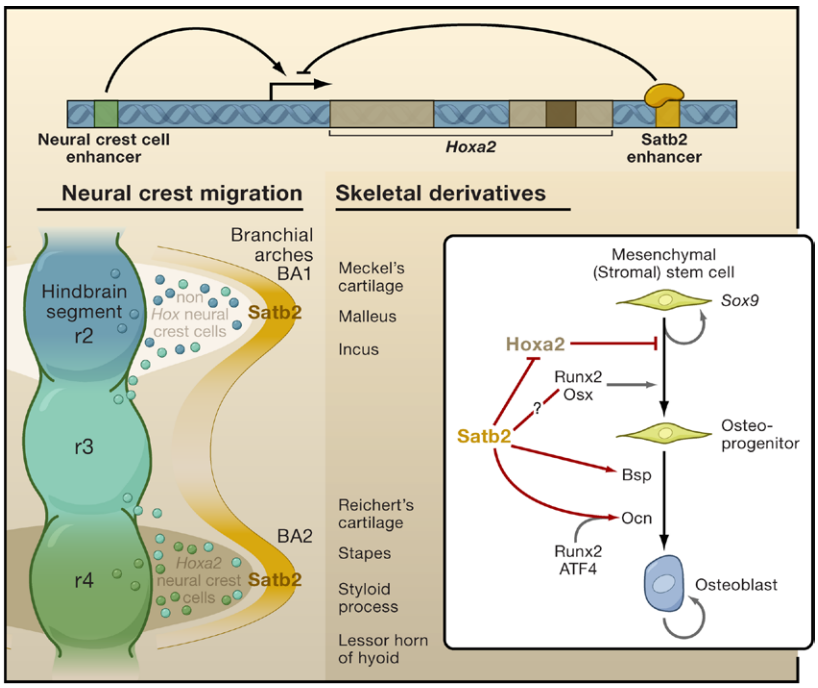

Figure 1. Satb2 in Bone Formation

Neural crest cells migrate from hindbrain segments (r2, r3, r4) into branchial arches 1 and 2 (BA1, BA2) of the developing mouse embryo. Neural crest cells expressing *Hoxa2* are found adjacent to the hindbrain r4 segment entering BA2 territory. The region of *Satb2* expression is shown in orange in branchial arches 1 and 2. The skeletal derivatives of the neural crest cells arise at later stages.

(Top) HoxA2 has roles in both patterning and antagonizing bone formation. An upstream enhancer regulates the expression of the *Hoxa2* gene in neural crest cells, whereas binding of Satb2 to a different enhancer element downstream of the gene suppresses *Hoxa2* expression.

(Inset) In addition to suppressing *Hoxa2* expression, Satb2 promotes production of Bsp and Ocn (the latter through an interaction with Runx2 and ATF4) to promote the differentiation of mesenchymal progenitors to osteoblasts.

ever, evidence is emerging for cross-talk between these processes. This is illustrated in studies that establish the functional role of *Hoxa2* in skeletal development. Experiments examining gain and loss of function of *Hoxa2* in several vertebrate species reveal reciprocal homeotic transformations of select craniofacial components, clearly demonstrating that it is a master regulator of skeletal patterning in craniofacial development (reviewed in Trainor and Krumlauf, 2001). In addition, *Hoxa2* has a separate role in antagonizing bone formation, as loss of *Hoxa2* causes an upregulation of *Sox9* and *Runx2* during osteoblast differentiation (Kanzler et al., 1998). Therefore, understanding the mechanisms that mediate these dual roles of *Hoxa2* will provide valuable insight into coordination of pathways governing bone patterning and differentiation. In this issue of *Cell*, Grosschedl and colleagues (Dobreva et al., 2006) make an important stride toward this goal by demonstrating that the nuclear matrix protein Satb2 represses *Hoxa2* expression and is an activator of multiple steps of Runx2-dependent osteoblast differentiation (Figure 1).

Satb1 and Satb2 comprise a family of nuclear matrix-attachment region (MAR) proteins. In eukaryotes, chromatin loops are generated by attachment of chromatin fibers to nonhistone chromosomal protein scaffolds within the nucleus, termed the nuclear matrix. Specific AT-rich DNA sequences at the base of looped-out chromatin domains are attachment sites for the nuclear matrix and are postulated to play important roles in regulating cell-specific gene expression. Transcriptional activity is dependent upon the state of chromatin condensation. Various models have proposed that decondensed chromatin loops out, thus facilitating the recruitment of transcription factors and stimulation of gene activity. In this regard, during T cell development, Satb1 is thought to regulate higher-order chromatin organization, modification, and gene transcription based on its ability to tether DNA elements and to act as a "landing platform" for several chromatin-remodeling complexes (such as Sin3a, CHRAC, and ACF) (Cai et al., 2003; Yasui et al., 2002). Satb2 interacts with MARs of the immunoglobulin μ locus in pre-B cells. Its ability to increase gene expression is dependent upon SUMO modifications mediated through lysine residues that differ from those in Satb1 (Dobreva et al., 2003). Additionally, in human development, haploinsufficiency of *SATB2* due to translocations involving 2q32-q33 correlates with the formation of a cleft palate.

Using targeted mutagenesis of *Satb2* in mouse, Dobreva et al. (2006) provide insight into how the nuclear matrix, chromatin remodeling, and gene activation come together to regulate osteoblast differentiation in development. Consistent with the involvement of human *SATB2* in cleft palate formation, the investigators found that *Satb2* regulates fusion of palatal shelves in the mouse. The most striking phenotypes detected in mice lacking Satb2 are craniofacial defects in skeletal elements and the inhibition of normal osteoblast differentiation. By combining an impressive series of molecular and genetic approaches, the authors reveal that *Hoxa2* is a critical target gene for repression by *Satb2* in the regulation of osteoblast differentiation (Figure 1). This is evidenced by the fact that bone formation defects are rescued in mouse embryos lacking both Satb2 and Hoxa2. Protein binding and regulatory analyses demonstrate that Satb2 binds to a consensus MAR site in an enhancer

3′ of the *Hoxa2* gene and that this motif is important for its regulatory activity. Therefore, *Hoxa2* is a direct transcriptional target of Satb2 in osteoblast differentiation. This raises the question of whether Satb2 regulates the function of *Hoxa2* in both patterning and differentiation or acts exclusively in osteoblast differentiation. It is interesting to note that the 3′ *cis*-regulatory module involved in mediating the Satb2 interaction is distinct from the 5′ cranial neural crest enhancer, which governs the role of *Hoxa2* in early anteroposterior patterning (Maconochie et al., 1999). Hence, distinct *cis*-regulatory modules are involved in directing aspects of *Hoxa2* expression in neural crest and osteoblast cells (Figure 1). It remains to be determined whether these enhancers have segregated or overlapping roles in the control of both patterning and differentiation.

In mutant embryos lacking *Satb2*, Dobreva et al. (2006) demonstrate by transcriptional profiling that a wide variety of transcription factors, extracellular matrix proteins, and metalloproteinase components important in bone formation are activated or repressed. Chromatin immunoprecipitation (ChIP) and transactivation experiments reveal that Satb2 can bind to and regulate bone sialoprotein (*Bsp*) and osteocalcin (*Ocn*) genes, themselves critical components in osteoblast formation. This strongly suggests that *Satb2* has multiple inputs into transcriptional control of osteoblast differentiation (Figure 1). Insight into another property of Satb2 arose from characterization of the *Ocn* response to Satb2. Unlike *Hoxa2* and *Bsp*, no consensus Satb2 binding sites could be detected in the *Ocn* locus, although ChIP assays with Satb2 show binding, thus suggesting indirect interactions. The osteoblast master regulators Runx2 and ATF4 are known to bind to specific nonadjacent regulatory modules in the *Ocn* locus, opening up the possibility of synergy between Satb2, Runx2, and ATF4 in regulating the expression of *Ocn*. On the basis of genetic synergy between mouse mutants, protein interaction and transactivation analyses, Dobreva et al. (2006) discovered that Satb2 has the ability to physically interact with Runx2 and ATF4 to stimulate cooperative binding and *Ocn* expression. This raises the exciting possibility that Satb2 not only serves as a platform for organizing chromatin-remodeling complexes in the nuclear matrix and itself modulates gene activity but may also assemble complexes with other DNA binding proteins to potentiate their activity. Interactions with Satb2 might stabilize weak interactions between activators, such as Runx2 and ATF4. Alternatively, activators may be tethered on a site in the nuclear matrix that enhances complex stability via additional protein-protein interactions.

The ability of MARs and nuclear matrix proteins to facilitate transcription and the assembly of complexes may be analogous to recent evidence in yeast that links active genes and nuclear pores. The recruitment of mRNA binding proteins or export factors to the promoters of transcriptionally active genes leads to a high affinity for the nucleopore-associated Sac3 protein, which brings the DNA transcription complex to the nuclear pore (Dilworth et al., 2005; Drubin et al., 2006). Hence, the nuclear pore provides a gateway for additional transcription factors to be recruited to specific sites of chromatin and for newly transcribed mRNA to be transported to the cytoplasm.

Distinguishing between the different modes of Satb2 activity will be important for a detailed understanding of how the nuclear matrix, chromatin structure, and transcriptional activity coordinate the regulation of multiple steps during osteoblast differentiation.

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